REMARKS

In response to the Final Office Action mailed on April 14, 2006, Applicant respectfully requests continued examination and reconsideration of this application and entry of the amendments and remarks submitted herewith.

Applicant thanks the Examiner for indicating that claims 5, 6, 21, 27 and 35 are allowable and for withdrawing the restriction between the polypeptide claims and some claims (15, 16, 26 and 28) reciting methods of use of the polypeptide (Group IV, drawn to a method of identifying agents which bind or modulate the activity of said kinase, classified in class 435, subclass 15). The Examiner declined to rejoin all claims drawn to the withdrawn subject matter (claims 12, 13 and 29-34). Herein, Applicant amends these withdrawn claims to attempt to address the reason for the Examiner's refusal to rejoin them. Applicant respectfully requests that claims 12, 13 and 29-34 be rejoined in view of the amendments.

Applicant also thanks Supervisor Jon Weber for helpful the discussion on June 15, 2006, regarding the finality of the office action. The method claims were discussed. Agreement was reached that a new office action would be provided, however, that did not occur. Applicant had concurrently filed a petition, because the deadline for that was reached; the petition also was discussed.

In the present response, claims 5, 12, 13, 15, 16, 26, 28, 29, 30 and 32 are being amended. Support for an amendment to claim 5 can be found in the specification, at, for example, paragraphs [00127] and [00142]. Support for another amendment to claim 5 and an amendment to claim 15 can be found in the specification, at, for example, paragraph [0088]. Support for the amendment to claim 16 can be found in the specification, at, for example, paragraph [00199]. Support for the amendment to claim 30 can be found in the specification, at, for example, paragraph [0043]. No new matter is being added. Claims 5, 6, 12, 13, 15, 16, 21, and 23-35 are pending. In the current Final Office Action, the Examiner has four outstanding concerns which are addressed herein.

Pages 3-4. Rejections of the Claims Under 35 U.S.C. §112, Second Paragraph
Claims 15, 16, 26 and 28 were rejected under 35 U.S.C. §112, second paragraph for allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Each rejection is treated separately in the following remarks.

Claim 15. Claim 15 was rejected under 35 U.S.C. §112, second paragraph for two reasons: a) reciting "a polypeptide of claim 5" and b) reciting "the activity".

In response to a), Applicant herein is amending claim 15 (and claim 12) to recite "the polypeptide of claim 5." In view of this amendment, Applicant respectfully requests that this rejection be withdrawn.

In part b), the Examiner appears to believe that the act of transferring a phosphate group to a substrate is the only definition of kinase activity. However, this is not how Applicant has presented the activities of the 14171 molecules of the invention. Applicant respectfully refers the Examiner to paragraph [0088] of the specification. In that paragraph, Applicant supports many kinase activities, from the submacromolecular level (e.g., activity ascribed to subdomain function, e.g. ATP binding, as a non-limiting example (also see paragraph [0076]) to the molecular level (e.g., the phosphorylation activity ascribed to domain and intact kinase function, as a non-limiting example) to the cellular level (e.g., modulation of cell death, as a non-limiting example). Furthermore, in Examples 3, 4, 11 and 12, Applicant exemplified multiple kinase activities for the polypeptides of the invention, including phosphorylation, reporter gene activation, and the ability to affect nuclear factor-κb. Further disclosure of such activities also is provided at, for example, paragraphs [0035]-[0037]. From these disclosures, it is clear that 14171 activities are more than the ability to transfer a phosphate group to a substrate. However, 14171 activities are well-defined and further exemplified in the application. In view of these remarks, Applicant respectfully requests this rejection be withdrawn.

Claims 16 and 28. Claims 16 and 28 were rejected as being indefinite for the recitation of "14171 kinase substrate." Specifically, the Examiner could not find a definition for this phrase. In the interest of furthering prosecution of this application, Applicant is amending these claims to recite a traceable definition. In view of these amendments, Applicant respectfully requests that this rejection be withdrawn.

Claim 26. Claim 26 was rejected as being indefinite for the logic of dependency and the function of ATP binding as a kinase activity. As discussed above, Applicant has provided support for ATP binding as an activity which could be studied to identify modulators of the polypeptides of the invention, as dependent from claim 15. However, Applicant has reconsidered the dependency and has adjusted the dependency of claim 26 to depend on claim 13, which Applicant requests to be rejoined.

The Examiner also complained about the concept of identifying modulators which compete with ATP for binding to a kinase. The Examiner claims that kinase modulators do not bind to the ATP-binding site, but instead bind to the catalytic site. Applicant herewith provides an Exhibit, a review article by Sebolt-Leopold and English, to provide some information to the Examiner. This article reviews, as examples of kinase modulators, a variety of kinase inhibitors. As can be seen in the left column of page 458 and the right column of page 460, many of these kinase modulators do bind to the ATP binding site and thus compete with ATP for binding.

In view of this amendment and these remarks, Applicant respectfully requests that this rejection be withdrawn.

CONCLUSIONS

The foregoing amendments and remarks are being made to place the Application in condition for allowance. Applicant respectfully requests reconsideration of the rejections and of the decision not to rejoin certain claims. Applicant requests the timely allowance of the pending claims because, in view of these amendments and remarks, Applicant respectfully submits that the rejections of claims 15, 16, 26 and 28 under 35 U.S.C. § 112 are herein overcome. Early notice to this effect is solicited.

If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is encouraged to call the undersigned.

This paper is being filed timely as a request for a three month extension of time is filed concurrently herewith. No additional extensions of time are required. In the event any additional extensions of time are necessary, the undersigned hereby authorizes the requisite fees to be charged to Deposit Account No. 501668.
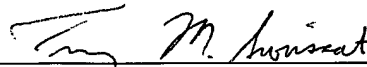
Entry of the remarks made herein is respectfully requested.

Respectfully submitted,

13 October 2006   MILLENNIUM PHARMACEUTICALS, INC.

By _____

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U.S. Serial No. 10/658,904, Filed September 10, 2003

**Exhibit Accompanying RCE and Amendment and Response after Final
to Office Action Dated April 14, 2006**

Exhibit	Sebolt-Leopold, Judith S. et al., "Mechanisms of drug inhibition of signalling molecules," NATURE, Vol. 441 (May 2006) pp 457-462

Mechanisms of drug inhibition of signalling molecules

Judith S. Sebolt-Leopold[1] & Jessie M. English[2]

The emergence of tumour-specific, molecularly targeted agents signifies a paradigm shift in cancer therapy, with less reliance on drugs that non-discriminately kill tumour and host cells. Although the diversity of targets giving rise to this new generation of anticancer drugs has expanded, many challenges persist in the design of effective treatment regimens. The complex interplay of signal-transduction pathways further complicates the customization of cancer treatments to target single mechanisms. However, despite uncertainty over precise or dominant mechanisms of action, especially for compounds targeting multiple gene products, emerging agents are producing significant therapeutic advances against a broad range of human cancers.

The elucidation of the signal-transduction network that drives neoplastic transformation has led to rationally designed cancer therapeutics that target specific molecular events. Over the past 20 years, we have witnessed an explosion in the launch of drug discovery programmes that, if successful, will significantly lessen our reliance on DNA-directed chemotherapeutic agents with low therapeutic indices as the mainstay of cancer treatment. However, targeted cancer drug candidates, independent of their mechanism of action, frequently face similar hurdles to those that challenge traditional agents. Problems of insufficient efficacy, development of resistance and unacceptable safety profiles continue to hamper clinical progress.

The successful development of molecularly targeted agents is all about making the right choices. Starting with initial target selection, the drug discovery process is fraught with critical junctures at which decisions are difficult. In addition, the stakes are high — each decision must be correct or success will remain elusive, or at best delayed. It all begins with target selection and betting on the right pathway (see the review in this issue by Lengauer and colleagues, page 451). Selection of the appropriate screening strategy for identifying inhibitors/activators around a given target can also be problematic — only to be followed by difficult choices over which 'hit' or chemical series a research team should focus their efforts on. As a drug discovery programme advances, preclinical model selection is often not straightforward. The predictability of animal models is a hotly debated topic with no imminent resolution[1,2]. The cycle of tough choices begins anew with the decision to advance a compound into the clinic. At this point, oncologists and trial sponsors must continue to make the right call on complex issues encompassing patient selection, treatment-regimen design, and the selection of appropriate biomarkers that measure patient response and address proof of concept, often for both the target and the compound selected for development. In the face of these formidable challenges, a number of agents are showing potential. Consequently, we seem to be on the right path in our attempts to treat cancer by disrupting the fundamental signalling pathways that tumours rely on to grow and survive. Here we describe rational points of pharmacological intervention in key signalling pathways. With a focus on kinase targets, this review is intended to provide mechanistic insights and to highlight special considerations that are inherent in the development of small-molecule inhibitors from both multi-targeted and truly selective classes of agents.

Exploiting the kinome

The c-src proto-oncogene was the first to be reported. Its discovery by Bishop and Varmus in 1976 (ref. 3) led to the finding that a 60-kDa phosphoprotein was the long-sought src gene product[4]. Shortly thereafter, two laboratories independently demonstrated that the Src protein was a kinase[5,6]. We now know that there are more than 50 different oncogenes, many of which are protein kinases[7]. The interconnecting role of proto-oncogenes in signalling cascades is finely tuned to maintain the growth and proliferation of normal cells. Upon oncogenic mutation or activation, aberrant cellular signalling drives subsequent tumorigenesis.

Among the first kinases to be identified and linked to tumorigenesis were receptor tyrosine kinases (RTKs). Upon binding of growth factor ligands, RTKs form both homodimers and heterodimers, resulting in activation of their intracellular kinase domains, and subsequent activation of downstream signalling cascades that stimulate proliferation and survival (Fig. 1). Agents targeting RTKs in oncology include therapeutic antibodies (that is, biological agents or biologics) to RTK ligands or the receptors themselves, and small-molecule inhibitors that target the intracellular kinase domains of the RTKs. Among the most aggressively targeted RTKs are the ErbB and vascular endothelial growth factor receptor (VEGFR) families of tyrosine kinases[8,9]. As depicted in Fig. 1, two major intracellular signalling cascades that are activated by tyrosine kinase receptors and co-opted in tumour cells are the Ras–mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3-OH kinase (PI(3)K)–AKT–mTOR (mammalian target of rapamycin) pathways (see the review in this issue by Shaw and Cantley, page 424).

The entire collection of kinases encoded by the human genome, which is known as the kinome and encompasses over 500 protein kinases, offers a rich and diverse source of potentially druggable targets for disrupting tumour growth and survival. Eight kinase-targeted oncology drugs have received regulatory approval so far (Table 1), and more than 100 additional agents are currently undergoing clinical evaluation. The diversity of kinases being targeted by these test small-molecule

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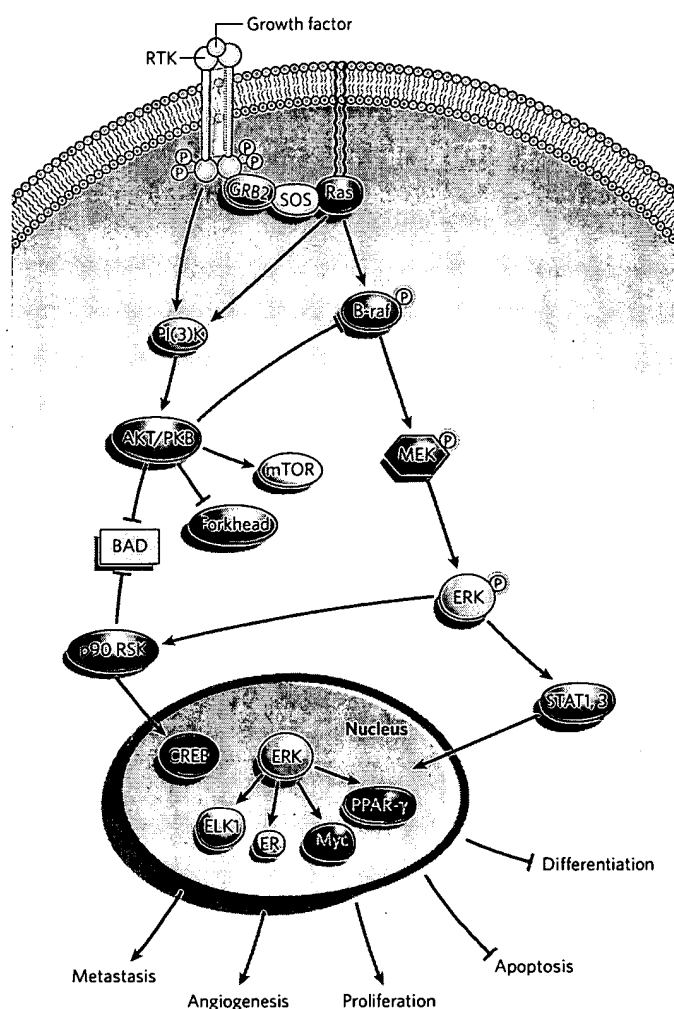
Figure 1 | Central growth factor signalling pathways that drive pleiotropic cellular responses. In tumour cells, a number of receptor tyrosine kinases (RTKs), which include the ErbB and vascular endothelial growth factor receptor families of tyrosine kinases, can become activated by various mechanisms, including mutation, overexpression and autocrine or paracrine production of their respective growth factor-family ligands. In response to ligand-induced activation of cell-surface RTKs, assembly complexes are formed that contain activated, autophosphorylated growth factor receptors with adaptor proteins such as growth factor receptor-bound-2 (GRB2) and exchange factors such as Son-of-sevenless (SOS), to activate Ras. Adaptor proteins are composed of Src homology 2 (SH2) and SH3 domains, which serve as docking sites for various signalling proteins, including receptors and GTPase regulators. In cooperation with GRB2, SOS activates Ras by catalysing the replacement of GDP with GTP. In its GTP-bound form, Ras initiates membrane recruitment and activation of Raf, leading to the activation of the dual specificity mitogen-activated protein kinase (MAPK) kinases MEK1 and MEK2. Activated MEK continues the cascade of phosphorylation events by activating MAPK/extracellular signal-regulated kinase-1 (ERK1) and ERK2. Once it is phosphorylated, ERK is translocated across the nuclear membrane, resulting in the activation of numerous transcription factors, including members of the ETS family that regulate cell-cycle progression (ELK1), as well as oestrogen receptor (ER), Myc, c-Fos, peroxisome proliferator-activated receptor-γ (PPAR-γ), and signal transducer and activator of transcription 1 (STAT1) and STAT3. Activated ERK also phosphorylates cytoplasmic p90 ribosomal protein S6 kinase (RSK), leading to phosphorylation and inactivation of the pro-apoptotic protein BAD. RSK activation further promotes cell survival by leading to the phosphorylation of cyclic-AMP-responsive element-binding protein transcription factor (CREB). Ras has a central role in mediating not only proliferation signalling through the MAPK pathway, but also survival signalling through the phosphatidylinositol-3-OH kinase (PI(3)K) pathway. Activated Ras interacts with PI(3)K to generate second-messenger lipids that are critical for activation of numerous target proteins, including the survival signalling kinase AKT/protein kinase B (PKB). AKT provides strong anti-apoptotic signals through its negative regulation of Raf, forkhead transcription factors and BAD. The PI(3)K–AKT pathway is also important in modulating mammalian target of rapamycin (mTOR), which is a serine/threonine kinase that acts as a central sensor for nutrient/energy availability, thereby regulating cell growth in response to the environment.

compounds or biologics is shown in Table 2. Of those agents in late-stage development, the majority target tyrosine protein kinases.

Modes of small-molecule inhibitor binding

The general architecture of a protein kinase is depicted in Fig. 2. Protein kinases possess a deep ATP-binding active site that also affords a high-affinity binding site for small-molecule inhibitors. Thus, many of the first kinase inhibitors were ATP mimetics that bound to this site and competed with cellular ATP. As the ATP site is highly conserved across the kinome, dogma suggested that it would be difficult to identify selective inhibitors that targeted only the therapeutically relevant kinase. It was feared that kinase inhibitors would be non-selective, thereby precluding the realization of a sufficient therapeutic index. Early evidence suggesting a path forward was provided by studies on isoforms of the p38 MAPK, indicating that residues close to the ATP-binding site could be exploited to achieve selectivity, even among closely related kinases[10,11]. Refinement continues with strategies that exploit amino-acid heterogeneity in, or close to, the ATP-binding site of kinases. Key to these efforts has been the elucidation of the human kinome combined with increasing numbers of solved kinase structures[12,13]. Most of the 75 or more kinase inhibitors that are currently in clinical trials seem to be ATP mimetic. However, the discovery of alternative binding modes for small-molecule inhibitors (Fig. 3) is being exploited to build a high degree of selectivity into drug molecules.

The first non-classical kinase inhibitors to be identified were the MAPK kinase (MEK) inhibitors PD98059 (ref. 14) and U0126 (ref. 15). It was recognized early on that these inhibitors were non-ATP-competitive and probably interacted with MEK1 and MEK2 in a unique way.

This novel binding mode seemed to confer desirable properties, such as enhanced selectivity relative to ATP-mimetic inhibitors[16]. Recently, this unusual binding mode was revealed in co-crystal structures demonstrating simultaneous binding of ATP and a close structural analogue of the MEK inhibitor CI-1040, confirming that these inhibitors are not ATP competitive[17]. The occupation of a unique inhibitor-binding pocket adjacent to the ATP site by CI-1040-like MEK inhibitors is thought to induce several conformational changes in unphosphorylated MEK, consequently serving to lock the enzyme in a closed but catalytically inactive form. Notably, the MEK inhibitor-binding pocket is located in a region where sequence similarity with other protein kinases is relatively low and distinct from the homologous ATP-binding site[17]. Therefore, nature has cooperated in providing drug researchers with an exploitable mechanism for developing highly selective inhibitors that block MEK-catalysed activation of the MAPK pathway.

As our database of small-molecule kinase inhibitors has expanded, additional exploitable binding modes have been revealed. Birb796, imatinib (Gleevec; STI571) and sorafenib (Nexavar; BAY 43-9006) bind in similar ways to the active site of p38α, Abl and B-raf kinases, respectively, extending beyond the highly conserved ATP-binding site[18–20] (see Fig. 3 for a depiction of Birb796 binding to p38). The elucidation of co-crystal structures of imatinib analogues with c-Abl, and Birb796 with p38, showed that these inhibitors take advantage of the conformational plasticity of kinases to constrain them in an inactive state. Whereas there are probably few conformations that a kinase can adopt to be active, they seem to have a range of conformations in the inactive state. Thus, the enhanced selectivity of these inhibitors derives, in part, from their binding to the inactive conformation of the kinase. The

Table 1 | Clinically approved kinase-targeted oncology agents

Drug	Number	Known targets
Small molecules		
Imatinib (Gleevec)	STI-571	Abl, PDGFR, c-Kit
Gefitinib (Iressa)	ZD-1839	EGFR
Erlotinib (Tarceva)	OSI-774/CP358774	EGFR
Sorafenib (Nexavar)	BAY 43-9006	VEGFR, PDGFR, FLT3, c-Kit, B-raf, Raf-1
Sunitinib (Sutent)	SU11248	VEGFR, PDGFR, FLT3, c-Kit
Biologics		
Trastuzumab (Herceptin)	–	ErbB2 (HER-2/neu)
Cetuximab (Erbitux)	–	EGFR
Bevacizumab (Avastin)	–	VEGF

Agents included in the table are approved by the Food and Drug Administration for use in the United States. Abl, Abelson leukaemia virus; EGFR, epidermal growth factor receptor; PDGFR, platelet-derived growth factor receptor; VEGFR, vascular endothelial growth factor receptor.

selectivity profile of these molecules cannot be predicted on the basis of highly conserved sequence similarity alone. Methods for predicting inhibitor cross-reactivity profiles are being developed[21]. As we enhance our understanding of the conformational plasticity of kinases, our ability to identify novel inhibitors that target the back pocket of kinases will be facilitated. In so doing, we will be able to further refine the selectivity profiles of target drug molecules.

Multi-targeted kinase inhibitors

As most cancers are the result of a number of mutations[22], it is reasonable to expect agents that target a number of different kinases to have a better chance of efficacy than highly selective kinase inhibitors administered as single agents. For decades, oncologists have taken advantage of combinations of agents with unique activities for the management of neoplastic disease. Combination therapy is the norm rather than the exception. However, it is difficult to rationally design small-molecule inhibitors that are highly potent and selective against a pre-determined array of desired kinase targets unless these targets are structurally highly similar. The discovery of multi-targeted kinase inhibitors has been largely empirical in the sense that many have evolved from drug discovery programmes in which non-selective ATP-competitive chemical matter was identified at the outset. Our understanding of cellular signalling and tumour genetics is not currently refined to the point that predictions can be successfully made regarding which combinations of kinase inhibition will yield maximal efficacy in a given tumour type. Therefore, most multi-targeted discovery programmes have two key laboratory objectives: first, to optimize activity against the critical targets; and second, to address the question of whether accompanying activity against another target is a positive attribute or a liability that needs to be designed out. Here we focus on two clinically successful multi-targeted kinase inhibitors — sunitinib (Sutent; SU11248) and sorafenib (Nexavar; BAY 43-9006) — to illustrate how distinctly different preclinical approaches led to approved agents with proven clinical efficacy against renal tumours.

Sunitinib emerged from a Sugen research programme that, from its early days, was focused on targeting the split kinase domain superfamily of RTKs. These proteins, encompassing VEGF family members as well as platelet-derived growth factor receptor-β (PDGFR-β), Kit and FLT3, are expressed on solid tumour cells, and participate in autocrine loops implicated in cancer growth and survival[23–25]. In addition, several of the split kinase domain RTKs, namely the VEGFRs and PDGFR-β, are key angiogenesis targets[26]. Thus, a multi-targeted kinase inhibitor capable of inhibiting all or several members of this family could potentially result in broad-spectrum anti-tumour efficacy. The subsequent demonstration that sunitinib possessed significant *in vivo* activity against a diverse panel of human xenografts provided further impetus for testing this hypothesis in the clinic[27]. The integration of biomarker readouts into these preclinical studies as biological indicators of drug function proved important in guiding the early clinical evaluation of this agent[27]. Pharmacokinetics

(PK; the time and dose dependence of the plasma drug concentration) were analysed relative to Sutent's pharmacodynamics (PD; the time and dose dependence of target modulation) and efficacy. By determining the PK–PD relationship, critical proof-of-concept criteria for a given drug candidate can be established before proceeding into early clinical trials. Importantly, an increase in VEGF-A and PDGF, and a concurrent decrease in sVEGFR-2, were subsequently shown in plasma samples analysed from patients treated with sunitinib[28]. sVEGFR-2, which is a soluble form of VEGFR-2, similar to sVEGFR-1 and other soluble circulating RTKs, is a potential quantitative biomarker of angiogenesis and anti-angiogenic drug activity[29]. Importantly, 40% of renal cancer patients treated with sunitinib were classified as partial responders, and the overall progression-free survival (PFS) of all patients under study was 8.7 months[28]. This robust clinical activity of sunitinib against metastatic renal cell carcinoma led to its recent regulatory approval for this indication. In addition, sunitinib has been approved for treatment of imatinib-refractory gastrointestinal stromal tumours (GISTs).

Sorafenib is also a multi-targeted kinase inhibitor, which was recently approved for treatment of metastatic renal cancer. However, in the early days of its preclinical development, the ability of sorafenib to inhibit VEGFR and angiogenesis was not fully appreciated. Early reports on sorafenib were exclusively devoted to accounts of its targeting of Raf-1, presumably by virtue of its inhibition of Raf kinase, which is a serine/threonine kinase central to the MAPK pathway[30,31]. A putative Raf-directed mechanism of action was consistent with sorafenib having been identified from high-throughput screening of small molecules against c-raf kinase. Crystallographic studies showed that sorafenib binds to the ATP pocket of B-raf, interacting with residues in both the P-loop and the kinase-activation loop. It is believed that inhibition of Raf catalytic activity by sorafenib is achieved by its ability to prevent the activation loop and the catalytic residues from adopting a conformation that is competent to bind and phosphorylate substrate[20]. After this agent entered clinical evaluation, the multi-targeted nature of sorafenib became widely known. As reported by Wilhelm and colleagues, this agent was also found to inhibit a number of RTKs involved in angiogenesis[32]. Most notably, it was shown to inhibit VEGFR-2, VEGFR-3 and PDGFR-β with roughly the same potency as wild-type and mutant B-raf protein kinases. Interestingly, it was also determined that sorafenib inhibits FLT3 and c-Kit[32]. Based on the clinical activity profile previously discussed for sunitinib, it is therefore not surprising that sorafenib also exhibited renal cancer activity leading to its regulatory approval for this indication. As summarized in the package insert for sorafenib, a 2.8-month prolongation in PFS (167 days versus 84 days for sorafenib-treated and placebo groups, respectively) was observed in a randomized phase III trial of renal cancer patients. The gain in PFS in sorafenib-treated patients primarily reflects the stable disease population, as the response rate was low (2%). Comparative biomarker data are needed to address whether the

Table 2 | The pipeline: representative kinase-directed oncology drug targets

Kinome branch	Compound	Known targets
TK	AZD-2171	VEGFR
	VandetanibZD6474	VEGFR, EGFR
	Vatalanib/PTK787	VEGFR, PDGFR, c-Kit
	Axitinib/AG-013736	VEGFR, PDGFR
	Lapatinib/GW572016	EGFR, ErbB2
	Dasatinib/BMS-354825	Src, Abl
	AMN-107	Abl
	CP-751871	Anti-IGFR antibody
CMGC	Flavopiridol	Pan-cdk
	PD332991	cdk4
STE	PD0325901	MEK
	ARRY-142886	MEK

Abl, Abelson leukaemia virus; cdk4, cyclin-dependent kinase-4; CMGC, cyclin-dependent kinase/mitogen-activated protein kinase/glycogen synthase kinase/cdk-like kinase; EGFR, epidermal growth factor receptor; IGFR, insulin-like growth factor receptor; MEK, mitogen-activated protein kinase kinase; PDGFR, platelet-derived growth factor receptor; STE, sterol; TK, tyrosine kinase; VEGFR, vascular endothelial growth factor receptor.
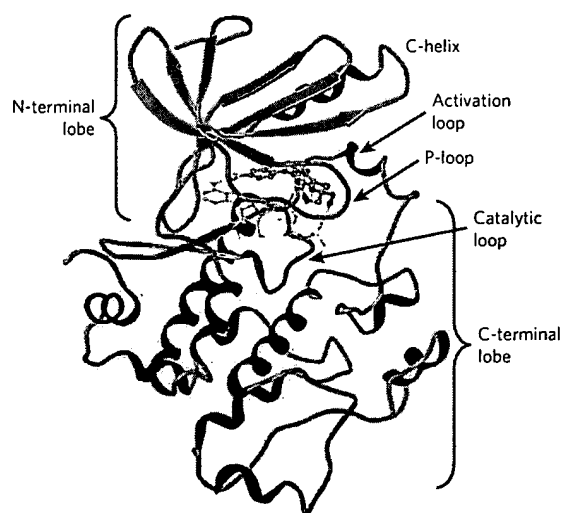
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Figure 2 | The protein kinase fold: ribbon diagram of MEK1 with bound ATP and PD318088. The architecture of mitogen-activated protein kinase kinase 1 (MEK1) is shown with the MEK1 inhibitor PD318088 and ATP bound simultaneously. Protein kinases form a conserved protein fold with an amino-terminal domain that primarily comprises β-strands, and a carboxy-terminal domain that is primarily α-helical. The ATP-binding and protein substrate-binding sites of protein kinases reside at the interface of these two domains. The highly conserved structural elements that are involved in regulating activity and catalysis are labelled. For many protein kinases, phosphorylation of the activation loop results in activation of the kinase and large conformational changes in the activation loop[57,58]. The P-loop is involved in alignment and binding of the phosphoryl groups of ATP. The proper alignment of the C-helix is required for productive binding of ATP and orientation of the active site into a productive mode. The C-helix is often misaligned in inactive kinases. The catalytic loop contains highly conserved residues required for phosphotransfer.

decreased response rate of sorafenib relative to sunitinib reflects differences in potency against VEGFR family members. It is unclear whether treatment with sorafenib results in clinically meaningful efficacy that is directly attributable to its inhibition of Raf kinase. As clinical trial data with sorafenib mature, it will be important to probe how well B-raf-mutated tumours respond to this agent.

It is generally difficult to ascertain the contribution of individual target activities when a multi-targeted kinase inhibitor shows clinical efficacy. However, preclinical studies can often provide clues. For example, studies reported by Bergers and colleagues[33] demonstrated that the combination of a VEGFR inhibitor that was structurally related to sunitinib, and imatinib, which inhibited PDGFR signalling, resulted in superior activity compared with VEGFR inhibitor alone. The two inhibitors target both tumour-associated pericytes via PDGFR, and endothelial cells via VEGFR[33]. Thus, the activity of sunitinib and sorafenib against renal cancer might be due, in part, to the ability of these agents to target both of these components of the tumour vasculature.

Highly selective kinase inhibitors

Antibodies, by virtue of the fact that they hit single targets, represent a rational approach for obtaining highly selective agents. The first protein kinase inhibitor to be approved was the monoclonal antibody trastuzumab (Herceptin), which targets the ErbB2 (HER-2/neu) receptor. This agent has subsequently become an important component of therapy for *HER-2*-positive breast cancer[34]. The monoclonal antibodies cetuximab (Erbitux) and bevacizumab (Avastin), targeting epidermal growth factor receptor (EGFR) and VEGF, respectively, have also gained regulatory approval[35,36]. Many more antibody-based clinical candidates are currently in clinical trials.

Another approach to the design of highly selective kinase inhibitors has focused on small molecules. Despite widespread scepticism, the ability to design highly selective ATP-mimetic kinase inhibitors has

now been demonstrated for a number of targets. Drug discovery programmes can take considerably longer when faced with the laboratory objective of designing out contaminating kinase activity. However, if successful, such programmes produce clean (that is, specific) inhibitors that serve as useful tools for probing the role of a given target in driving the phenotype of individual tumours. Consequently, such agents are potentially amenable to providing customized therapy to cancer patients. The approved agents gefitinib (Iressa; ZD-1839) and erlotinib (Tarceva; OSI-774) are examples of highly selective ATP-competitive kinase inhibitors targeting EGFR[37,38]. Other examples include lapatinib, which has dual affinity for ErbB2 and EGFR, and the highly selective cyclin-dependent kinase-4 (cdk4) inhibitor PD0332991, both of which are currently the subject of clinical trials[39,40].

Historical screening programmes directed towards protein kinase targets have often used recombinant catalytic kinase domains. It is therefore hardly surprising that the majority of small-molecule inhibitors reported so far are ATP-competitive in nature. The tendency to triage a large collection of hits on the basis of potency alone adds to the likelihood of starting out with highly promiscuous chemical matter. As discussed previously, exceptions are non-classical MEK inhibitors, which are highly selective by virtue of their binding to a unique pocket that is adjacent to, but distinct from, the ATP-binding site[17] (Fig. 3).

Truly selective kinase inhibitors can serve as useful tools for probing the role of a given target and its pathway in various cellular events. The MEK inhibitor PD98059, for example, was released to the academic community, where its usefulness is reflected by its citation in more than 4,000 publications. Single-targeted agents, encompassing both monoclonal antibodies and small-molecule inhibitors, are also potentially useful for the design of combination treatments specifically tailored to known genetic defects of a given tumour. The high incidence of B-raf mutations in melanomas[41,42], for example, suggests that they would be highly susceptible to treatment with MAPK pathway inhibitors — for example, MEK or B-raf inhibitors. This prediction was borne out preclinically, as reported by Solit and colleagues for B-raf-mutated melanoma xenografts treated with the MEK inhibitor PD0325901 (ref. 43). However, multiple genetic defects and tumour heterogeneity might make it unlikely that monotherapy with highly selective agents will be sufficient to eradicate tumour burden. The outcome of current clinical testing of the MEK inhibitors PD0325901 and ARRY-142886 (refs 44, 45) in melanoma patients should enhance our understanding of whether this potential challenge translates into a real concern.

Resistance to kinase inhibition

The emergence of drug resistance in treated patients has become a significant issue, and occurs in response to treatment with many approved agents, including imatinib, gefitinib and erlotinib. In some
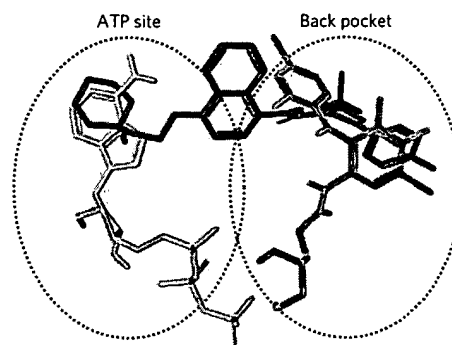

Figure 3 | Alternative binding modes of kinase inhibitors. The binding modes of two kinase inhibitors are shown in relation to the binding site of ATP (grey) in the kinase active site. The MEK1 inhibitor PD318088 (cyan) binds simultaneously with ATP in a region of the kinase active site that is adjacent to the ATP-binding site. Birb796 binding to p38 (purple) extends into the ATP site, but also accesses this back pocket, partly overlapping the region where PD318088 binds in MEK1.

cases, amplification of the oncogenic protein kinase gene can confer resistance[46]. Frequently, however, mutations occur in the kinase catalytic domain, and confer resistance by directly preventing or weakening interaction of the protein with the drug. It is not uncommon to find that drug-resistant mutations in breakpoint cluster region (Bcr)–Abelson leukaemia virus (Abl), Kit and EGFR, are structurally related by virtue of homologous mutations of a conserved 'gatekeeper' threonine residue[47,48]. The clustering of point mutations near protein kinase active sites has led to the search for new therapies against mutated targets resistant to first-line inhibitors. Certain drug-resistant mutants are not necessarily refractory to inhibition with an agent directed against the ATP site of the protein. Examples include the dual Src/Abl kinase inhibitor dasatinib (BMS-354825) and the imatinib derivative AMN107, both of which are effective against imatinib-resistant Abl kinase[49]. Another approach for discovering new agents that are effective against resistant tumours is the employment of alternative *de novo* screening approaches that are less likely to deliver traditional ATP-competitive chemical leads[50]. However, resistance to non-ATP-competitive kinase inhibitors, as observed with the MEK inhibitor CI-1040, can develop in the absence of any mutational changes to the protein, presumably due to changes in expression of other key pathway regulatory molecules[51]. It is therefore critical that we anticipate the development of drug resistance when designing novel combination-therapy strategies. For example, *a priori*, the combination of two highly selective agents acting within the same signalling pathway would probably not offer any therapeutic benefit over monotherapy with either agent. However, on a longer-term basis, an advantage might be seen, as manifested by a decreased incidence of resistance to pathway inhibition upon combination of the two agents. In the case of a multi-targeted kinase inhibitor, which can be viewed as combination therapy in a single pill, the development of resistance might be delayed if inhibition of multiple targets contributes to its anti-tumour effects.

Patient selection and design of rational drug combinations

Molecularly targeted therapy will have truly arrived when patients are matched with a treatment regimen predicated by their genetic attributes, as opposed to the histological classification of their malignancies. Gene-expression signatures might prove powerful in determining the extent to which multiple signalling pathways are activated[52]. The design of combination regimens customized to the tumours of individual patients is clearly the correct course and the ultimate goal of current research. As the Ras–MAPK and PI(3)K pathways are strongly interconnected, disruption of one will, in many cases, push tumour cells to increase flux through the other in a virtual tug of war between proliferation and survival signals. Combined inhibition of EGFR and PI(3)K signalling in phosphatase and tensin homologue (PTEN)-mutated tumours has been shown to be a rational and therapeutically advantageous approach[53]. A number of rational drug combinations can be envisioned that address tumour heterogeneity by targeting different processes or signalling pathways within tumours. There is a common misperception that the individual agents employed in combination trials must first be approved before clinical testing can proceed. However, phase I/II studies of bevacizumab tested in combination with erlotinib for the treatment of non-small-cell lung cancer and renal cancer were conducted prior to the approval of either agent[54,55]. While intellectual property issues can hinder expedient combination testing of agents that are not solely under the control of a single sponsor, channels nonetheless exist for proceeding with these critical clinical studies. In addition to this 'designer cocktail' approach, we should also continue to explore the efficacy of molecularly targeted signalling agents in combination with conventional chemotherapeutic drugs, especially when there is a strong scientific rationale[56].

Looking forward

Despite our advances in understanding the molecular events that are central to tumorigenesis, only eight kinase-targeted oncology drugs have been approved since 1998. While these statistics reflect incremental progress, there is reason for guarded optimism, as the number and quality of agents currently undergoing clinical evaluation are relatively high. Yet success will require persistence. Kinases are centre stage in the dysregulation of tumour growth and survival. Although failed agents might come and go, these important protein targets remain a constant feature that must be disrupted if we are to succeed in developing more effective cancer therapies. Consequently, we should be receptive to the need for multi-targeted, as well as single-targeted, kinase inhibitors. There is plenty of therapeutic opportunity for both, and often the distinction separating these two functional classes is ambiguous. For example, imatinib could be viewed as a multi-targeted agent due to its ability to inhibit Abl kinase, c-Kit and PDGFR. Hence, this agent is efficacious against both chronic myelogenous leukaemia (CML) and GIST. However, from the perspective of a CML cell, imatinib probably acts as a single-targeted agent due to the causative role of Bcr–Abl in driving its malignancy. As exemplified by sorafenib, the development of some agents might start out with the belief that they selectively hit one target, but end with the realization that they target multiple kinases, several of which might be important to activity. Semantics notwithstanding, there is a clear need to assemble a collection of pharmaceutically attractive kinase inhibitors that have the proven ability to influence key pathways and to modulate their respective targets at doses that are clinically achievable. Only then will we be in a position to impair multiple pathways non-empirically by judicious combination of selective agents. With that goal in sight, it is imperative that clinical investigators and trial sponsors alike have confidence in these targets and persevere. ∎

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